

PRECLINICAL STUDIES

Role of Angiotensin II Type 2 Receptors and Kinins in the Cardioprotective Effect of Angiotensin II Type 1 Receptor Antagonists in Rats With Heart Failure

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OBJECTIVES	We studied the role of angiotensin II type 2 (AT ₂) receptors and kinins in the cardioprotective effect of angiotensin II type 1 antagonists (AT ₁ -ant) in rats with heart failure (HF) after myocardial infarction.
BACKGROUND	The AT ₁ -ant is as effective as angiotensin-converting enzyme inhibitors in treating HF, but the mechanisms whereby AT ₁ -ant exert their benefits on HF in vivo are more complex than previously understood.
METHODS	Brown Norway Katholiek rats (BNK), which are deficient in kinins because of a mutation in the kininogen gene, and their wild-type control (Brown Norway [BN]) underwent myocardial infarction. Two months later, they were treated for two months with: 1) vehicle; 2) AT ₁ -ant (L158809, Merck, Rahway, New Jersey); 3) AT ₁ -ant + AT ₂ -ant (PD-123319, Parke Davis, Ann Arbor, Michigan); or 4) AT ₁ -ant + kinin B ₂ receptor antagonist (B ₂ -ant) (icatibant) (only BN). We measured left ventricular weight (LVW) gravimetrically, myocyte cross-sectional area (MCSA) and interstitial collagen fraction (ICF) histologically, and ejection fraction by ventriculography.
RESULTS	Development of HF was comparable in BN and BNK rats. The AT ₁ -ant reduced LVW and MCSA and the AT ₂ -ant blocked these effects in BN rats, but the B ₂ -ant did not. The AT ₁ -ant reduced LVW and MCSA in BNK rats, and this effect was reversed by the AT ₂ -ant. In BN rats, ICF was reduced and LVEF increased by AT ₁ -ant, and both AT ₂ -ant and B ₂ -ant reversed these effects. In BNK rats, the AT ₁ -ant failed to reduce ICF, and its therapeutic effect on LVEF was significantly blunted.
CONCLUSIONS	In HF, the AT ₂ receptor plays an important role in the therapeutic effects of AT ₁ -ant, and this effect may be mediated partly through kinins; however, kinins appear to play a lesser role in the antihypertrophic effect of AT ₁ -ant. (J Am Coll Cardiol 2004;43:1473–80) © 2004 by the American College of Cardiology Foundation

Myocardial infarction (MI) induces left ventricular (LV) dilatation associated with myocyte hypertrophy and interstitial fibrosis of the noninfarcted myocardium. These changes in LV geometry, referred to as remodeling after MI, contribute to the development of heart failure (HF) (1). Activation of vasoactive hormones, such as angiotensin, kinins, and nitric oxide, may be an important adaptive and/or maladaptive mechanism in the pathophysiology of HF. There is evidence that the renin-angiotensin system is activated in HF. Angiotensin II (Ang II), the key effector peptide of the renin-angiotensin system, has been implicated in the development of ventricular remodeling through its proposed growth-promoting properties, thereby stimulating myocyte hypertrophy and collagen synthesis, which lead to myocardial dysfunction. Angiotensin II receptors

comprise two major subtypes, angiotensin II type 1 (AT₁) and angiotensin II type 2 (AT₂) (2). Most known effects of Ang II in the cardiovascular system are mediated by the AT₁ receptor. It has been reported that the AT₂ receptor is involved in inhibition of cellular differentiation, growth, and apoptosis and may be activated in disease states such as MI and cardiac hypertrophy (3–5). Thus, the AT₂ receptor could play an important role in counterbalancing some of the effects of Ang II by antagonizing its action on the AT₁ receptor. Angiotensin II type 1 antagonists (AT₁-ant) have been shown to improve cardiac function and remodeling in HF in both clinical and animal studies (6,7). It has been proposed that the blockade of AT₁ receptors increases Ang II, activating AT₂ receptors, which leads to cardioprotection either directly or via kinins (8), although their exact physiological or pathophysiological role is not clearly defined.

Kinins, which are potent vasoactive peptides, are released from high- and low-molecular-weight kininogens by plasma and tissue kallikreins (9). Evidence suggests that a local kallikrein-kinin system exists in the heart, which enables it

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Abbreviations and Acronyms

Ang II	= angiotensin II
AT ₁	= angiotensin II type 1
AT ₁ -ant	= angiotensin II type 1 antagonist
AT ₂	= angiotensin II type 2
B ₂ -ant	= kinin B ₂ receptor antagonist
B ₂ -KO	= B ₂ kinin receptor knockout
BN	= Brown Norway
BNK	= Brown Norway Katholiek
EDV	= end-diastolic volume
EF	= ejection fraction
ESV	= end-systolic volume
HF	= heart failure
ICF	= interstitial collagen fraction
LV	= left ventricular/ventricle
LVW	= left ventricular weight
MBP	= mean blood pressure
MCSA	= myocyte cross-sectional area
MI	= myocardial infarction
NO	= nitric oxide

to synthesize and release kinins (10,11). Kinins act via two receptors, B₁ and B₂, and most of their effects have been attributed to the B₂ receptor, although recent evidence suggests that the B₁ receptor may also play an important role (12–14). Several studies have shown that during acute myocardial ischemia, release of kinins from the heart is rapidly increased. We have previously reported that blood pressure and severity of myocardial ischemia-reperfusion injury in Brown Norway Katholiek (BNK) rats (which are deficient in kinins because of a mutation in the kininogen gene) and in B₂ kinin receptor knockout (B₂-KO) mice were no different than their wild-type controls (15); however, they exhibited a diminished response to ischemic preconditioning (15). We further reported that in BNK rats and in B₂-KO with chronic HF due to MI, infarct size and development of HF were no different than their respective controls; however, the effects of angiotensin-converting enzyme inhibitors were diminished in BNK rats and B₂-KO mice with HF after MI (16,17). These studies suggest that although kinins may not be important in either cardiac remodeling or the pathophysiology of HF under basal conditions, in response to angiotensin-converting enzyme inhibitors they play an important role in decreasing remodeling and improving cardiac function.

In the present work, we tested the hypothesis that in rats with HF after MI, the effects of AT₁-ant on cardiac remodeling and LV function are mediated in part by the AT₂ receptor. Furthermore, we hypothesized that some of the effects of the AT₂ receptor are mediated by kinins whereas others are not. To study the role of the AT₂ and B₂ receptors, we used an AT₂-ant and a B₂ receptor antagonist (B₂-ant), respectively. To determine the role of kinins, we used kinin-deficient BNK rats, expecting that in this strain those effects of AT₁-ant that are mediated by kinins via both the B₁ and/or B₂ receptors would be decreased or absent,

whereas those effects that are mediated by activation of the AT₂ receptor independently of kinins should be present.

MATERIAL AND METHODS

Male BNK rats (obtained from the Department of Pharmacology, Kitasato University School of Medicine, and bred in our animal care facilities) and Brown Norway (BN) rats (Charles River Laboratories, Wilmington, Massachusetts), both weighing 255 to 275 g, were housed in an air-conditioned room with a 12-h light/dark cycle where they received standard laboratory rat chow (0.4% sodium) and drank tap water. They were given five to seven days to adjust to their new environment and were anesthetized with sodium pentobarbital before all surgical procedures (50 mg/kg intraperitoneally). This study was approved by the Henry Ford Hospital Institutional Animal Care and Use Committee.

Experimental HF model. Coronary artery ligation was performed as described previously (18). Rats were intubated and ventilated with room air using a positive-pressure respirator (Model 680, Harvard, South Natick, Massachusetts). A left thoracotomy was performed via the fourth intercostal space and the lungs retracted to expose the heart. After opening the pericardium, the left anterior descending coronary artery was ligated with a 7-0 silk suture near its origin; acute myocardial ischemia was deemed successful when the anterior wall of the LV became cyanotic. The lungs were inflated by increasing positive end-expiratory pressure, and the thoracotomy site was closed in layers. Another group of rats underwent sham ligation; the procedure was similar, but the suture was not tightened around the coronary artery. Our previous study showed that rats developed HF (ejection fraction [EF] 30% to 35%) by two months after a large MI (19).

Experimental protocols. Two months after coronary ligation, BN and BNK rats were randomly divided into the following groups:

1. Vehicle: BN (n = 11) and BNK (n = 17) were treated with vehicle (tap water) for two months.
2. AT₁-ant (L158809, Merck): BN (n = 17) and BNK (n = 17) were treated for two months with the AT₁-ant (1.5 mg/kg/day) in drinking water. To calculate how much AT₁-ant we should add to the water, we estimated intake at 20 ml/day.
3. AT₁-ant + AT₂-ant (PD123319, Parke Davis, Ann Arbor, Michigan): BN (n = 6) and BNK (n = 5) were treated with AT₁-ant + AT₂-ant (10 mg/kg via mini-osmotic pump).
4. AT₁-ant + B₂-ant (icatibant): BN (n = 16) with HF were treated with AT₁-ant + B₂-ant (100 µg/kg/day via mini-osmotic pump). We did not use the B₂-ant in BNK because they are deficient in kinins.
5. BN and BNK sham ligation: BN (n = 10) and BNK (n = 12).

Cardiac hemodynamics and ventriculography. Rats were anesthetized and a polyethylene catheter (PE-50) inserted into the carotid artery. Mean blood pressure (MBP) and heart rate were measured with a P23XL pressure transducer connected to a processor (Gould Brush 220, Cleveland, Ohio). The carotid catheter was advanced into the LV and ventriculograms recorded on 35-mm cine film at 60 frames/s during injection of 0.5 ml contrast material (Renom-60, Squibb, New Brunswick, New Jersey). The films were projected, and the margins of the LV image traced during end-diastole and end-systole over three consecutive cardiac cycles. End-systolic volume (ESV) and end-diastolic volume (EDV) were determined using the area-length method (20). Left ventricular ejection fraction (EF) was calculated as: $EF = (EDV - ESV)/EDV$.

Pathological and histological examination. After the hemodynamic and ventriculographic studies, the rats were killed and the chest opened. The heart was stopped during diastole by injecting 15% potassium chloride, and then excised and weighed and the LV sectioned transversely into four slices from apex to base. Slices were rapidly frozen in isopentane solution pre-cooled in liquid nitrogen and used for morphometric analysis of infarct size and histological examination. The histological study only involved the non-infarcted myocardium.

Determination of myocardial infarct size. A 6- μ m section from each slice was stained with Masson's trichrome. Sections from all four slices were projected onto a screen. Infarct size was determined by Gomori trichrome staining and expressed as the ratio of the infarcted portion to total LV circumference (21).

Determination of myocyte cross-sectional area (MCSA) and interstitial collagen fraction (ICF). Sections were pretreated with 3.3 U/ml neuroaminidase type V (Sigma, St. Louis, Missouri), then double-stained with: 1) fluorescein-labeled peanut agglutinin (Victor Lab, Burlingame, California) to delineate MCSA and the interstitial space; and 2) rhodamine-labeled *Griffonia simplicifolia* lectin I to show only the capillaries. From each slice, four radially oriented microscopic fields were selected at random and photographed at a magnification of 400 times. Myocyte cross-sectional area was measured by computer-based planimetry (Jandel Scientific, Corte Madera, California), ICF was calculated as total interstitial space minus total capillary area, and MCSA and ICF from all photographs were averaged (8).

Data analysis. All data are expressed as mean \pm SEM. Student's *t* test was used to compare mean values of the various parameters (left ventricular weight [LVW], MCSA, ICF, infarct size, MBP, LVEF, LVEDV, and LVESV) among different groups, specifically, sham versus HF-vehicle, HF-vehicle versus HF-AT₁-ant, HF-AT₁-ant versus HF-AT₁-ant + AT₂-ant, and HF-AT₁-ant versus HF-AT₁-ant + B₂-ant in BN and BNK rats. Hochberg's method for multiple comparisons was used to adjust the alpha level of significance (22). Effects of AT₁-ant treat-

ment in the two types of rats (BN and BNK) were compared using two-way analysis of variance, as were differences from HF-vehicle to HF-AT₁-ant between BN and BNK. A test for interaction was used between group and strain. A significant result would indicate that the effect observed between groups is dependent upon the strain.

RESULTS

Of the 52 BN rats that underwent left anterior descending coronary artery ligation, 1 died after surgery, for a mortality rate of 2%. In addition, one of the BN rats in the HF-vehicle group died within three months. Of the 41 BNK rats that underwent coronary ligation, 2 of those in the HF-vehicle group died within three months after MI. No rats in the sham-ligated group died.

Infarct size. Infarct size was similar in all groups (Table 1). All rats with coronary ligation developed a large MI, ranging from 40% to 45% of the LV circumference.

Heart weight, MCSA and ICF in BN and BNK rats during the development of HF. Left ventricular weight, MCSA, and ICF were similar in sham-ligated BN and BNK rats. Left ventricular weight, ICF, MCSA, LVEDV, and LVESV increased, and LVEF decreased similarly in the HF-vehicle group in both strains (Fig. 1). Thus, both BN and BNK rats developed similar remodeling and HF after MI.

Effect of AT₁-ant and B₂-ant on heart weight, MCSA, and ICF in BN and BNK rats. In BN rats, the AT₁-ant significantly reduced LVW ($p < 0.001$ vs. HF-vehicle) and MCSA ($p < 0.001$ vs. HF-vehicle), while the AT₂-ant reversed these effects. In BNK rats, the AT₁-ant also decreased LVW ($p < 0.05$ vs. HF-vehicle) and MCSA ($p < 0.001$ vs. HF-vehicle) and, again, the AT₂-ant blocked these effects ($p < 0.05$ vs. HF-AT₁-ant) (Fig. 2). In BN rats, the B₂-ant did not reverse the effect of the AT₁-ant on either LVW or MCSA. Although these effects seemed less evident in BNK than in BN, the differences were not statistically significant (Fig. 3). These data on LVW and MCSA suggest that AT₂ receptors play an important role in the antihypertrophic effect of AT₁-ant in rats with HF and that this effect is independent of kinins.

In BN rats, the AT₁-ant significantly decreased ICF ($p < 0.01$ vs. HF-vehicle), and this decrease was reversed by both AT₂-ant ($p < 0.001$) and B₂-ant ($p < 0.001$). In BNK rats, the AT₁-ant did not significantly decrease ICF, and the AT₂-ant had no effect (Fig. 2). The decrease in ICF was significantly greater in BN than in BNK rats (Fig. 3). These results suggest that the antifibrotic effect of the AT₁-ant was partly mediated by kinins via the B₂ receptor.

Hemodynamics and cardiac function during the development of HF. Sham-ligated BN and BNK exhibited no significant differences in MBP, LVEDV, LVESV, or LVEF. In both HF-vehicle groups LVEDV and LVESV were increased and MBP and LVEF were decreased, but these changes were similar in both strains (Table 1, Fig. 1).

Table 1. Hemodynamics, Infarct Size, and Heart Weight in BN and BNK Rats

	BN					BNK			
	Sham (n = 10)	HF-Vehicle (n = 11)	HF-AT _{1a} (n = 17)	HF-AT _{1a} + AT _{2a} (n = 6)	HF-AT _{1a} + B _{2a} (n = 16)	Sham (n = 12)	HF-Vehicle (n = 17)	HF-AT _{1a} (n = 17)	HF-AT _{1a} + AT _{2a} (n = 5)
BW (g)	354 ± 7	368 ± 10	338 ± 5‡	324 ± 12	332 ± 6	332 ± 6	381 ± 11	335 ± 5§	337 ± 10
MBP (mm Hg)	109 ± 6	108 ± 5	92.4 ± 3‡	87 ± 6	74 ± 3.5§	122 ± 5	117 ± 4	78.5 ± 3§	90 ± 8
HR (beats/min)	386 ± 10	371 ± 11	383 ± 7	350 ± 11	370 ± 12	385 ± 10	357 ± 14	350 ± 10	364 ± 23
IS (% of LV)	—	45 ± 3	40.3 ± 2	45 ± 3	42 ± 2	—	40 ± 2	41 ± 2	43 ± 1
HW (mg/100 g BW)	251 ± 7.5	324 ± 12*	264 ± 5§	314 ± 14§	267 ± 7	261 ± 7	306 ± 9*	283 ± 7‡	323 ± 14‡
RV (mg/100 g BW)	45.4 ± 1.8	58.5 ± 5.5*	49.1 ± 1.1†	58.9 ± 5	54 ± 2	49 ± 3	56.5 ± 2.3*	53.5 ± 1.3	62.7 ± 4.2†

Each value represents mean ± SEM. *p < 0.001, vs. sham. †p < 0.05, ‡p < 0.01, §p < 0.001 vs. HF-vehicle; †p < 0.05, §p < 0.001 vs. HF-AT_{1a}.

AT_{1a} = angiotensin II type 1 antagonist; AT_{2a} = angiotensin II type 2 antagonist; B_{2a} = kinin B₂ receptor antagonist; BN = Brown Norway; BNK = Brown Norway Katholiek; BW = body weight; HF = heart failure; HR = heart rate; HW = heart weight; IS = infarct size; LV = left ventricle; MBP = mean blood pressure; RV = right ventricle.

Effect of AT₁-ant and B₂-ant on MBP, LVEDV, LVESV, and LVEF. The AT₁-ant reduced MBP in both strains (BN: p < 0.01; BNK: p < 0.001 vs. HF-vehicle). In BN rats, the AT₁-ant significantly decreased EDV (p < 0.01) and ESV (p < 0.001) and increased LVEF (p < 0.001) compared with HF-vehicle, while the AT₂-ant reversed these effects (Fig. 2). The B₂-ant partially but significantly reversed the effect of ESV (p < 0.01) and EF (p < 0.001) and marginally reversed the effects of the AT₁-ant on LVEDV (p < 0.05) (Fig. 4). In BNK rats, the AT₁-ant caused a very small decrease in LVEDV (p > 0.05), and the same was true of LVEF (p > 0.05). Changes in LVESV and LVEF were much smaller in BNK than in BN rats (Fig. 4, right panel), and the AT₂-ant had no effect (Fig. 2). These data suggest that the AT₂ receptor participates significantly in the beneficial effects of AT₁-ant, which are mediated to some extent by kinins.

DISCUSSION

We found that in rats genetically deficient in kininogen, the precursor of kinins, basal cardiac hemodynamics and function, as well as morphology and histology, were no different from rats with an intact kallikrein-kinin system. Kinin deficiency did not aggravate cardiac remodeling after MI; indeed, infarct size, as well as LV hypertrophy, ICF, LVEDV, LVESV, and LVEF, were all similar in both kininogen-deficient BNK rats and BN rats with normal kininogen, suggesting that either kinins do not play a major role in the regulation of cardiac structure and function or that BNK rats have developed a compensatory mechanism to overcome the lack of circulating kinins. We further determined that the AT₂ receptor participates in the therapeutic effects of AT₁-ant because its effects were blocked by the AT₂-ant. Kinins and the B₂ receptor were found to participate in some but not all of the cardioprotective effects of the AT₁-ant because the decreased ICF and improved LVEF, EDV, and ESV caused by the AT₁-ant were blocked by the B₂-ant in BN rats and entirely absent in BNK rats. Collectively, these data suggest that the beneficial cardiac effects of AT₁-ant on HF are mediated at least in part by the AT₂ receptor via kinins.

Our data demonstrated that the decrease in heart weight caused by AT₁-ant was greater in BN than in BNK rats (BN: −60 mg/100 g body weight; BNK: −23 mg/100 g body weight; p < 0.05). Also, the AT₁-ant decreased both LVW and MCSA in BNK rats; however, these changes, although statistically significant, tended to be smaller than in BN rats. Furthermore, the antihypertrophic effects observed in BN rats were blocked by the AT₂-ant but not by the B₂-ant. We have previously found that in Lewis rats with HF due to MI, the B₂-ant did not block the antihypertrophic effect of the AT₁-ant (8). Similarly, in B₂-KO mice with HF after MI, we found that the AT₁-ant decreased LV weight, MCSA, and LV mass, although the last of these decreases was less pronounced in B₂-KO mice

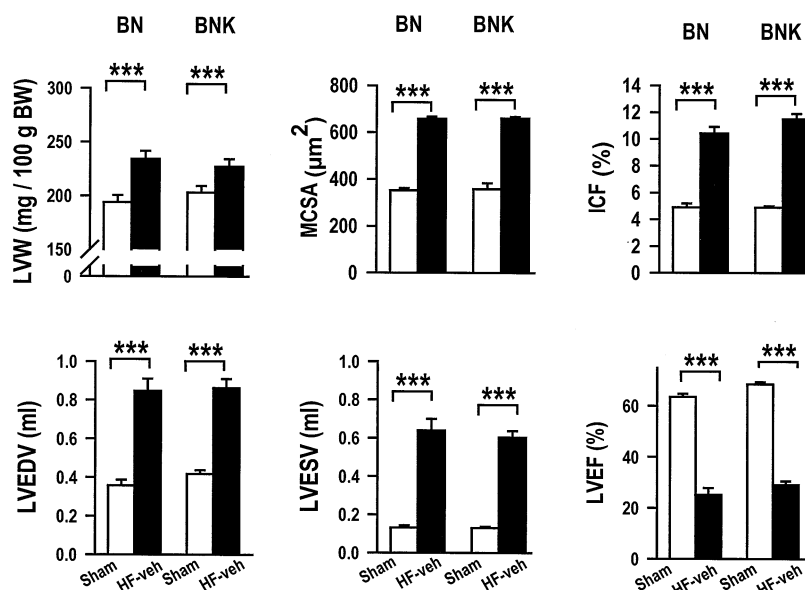


Figure 1. Left ventricular weight (LVW), myocyte cross-sectional area (MCSA), interstitial collagen fraction (ICF), left ventricular end-diastolic volume (LVEDV), left ventricular end-systolic volume (LVESV), and left ventricular ejection fraction (LVEF) four months after sham or coronary artery ligation in Brown Norway (BN) and Brown Norway Katholiek (BNK) rats. ****p* < 0.001. HF = heart failure.

than in their wild-type controls. Taken together, these data suggest that most of the antihypertrophic effects of AT₁-ant are not mediated by kinins.

Finally, we found that the effects of AT₁-ant are inde-

pendent of the reduction in blood pressure; although its effects on cardiac function and remodeling were accompanied by a reduction in MBP, the AT₂-ant or kinin antag-

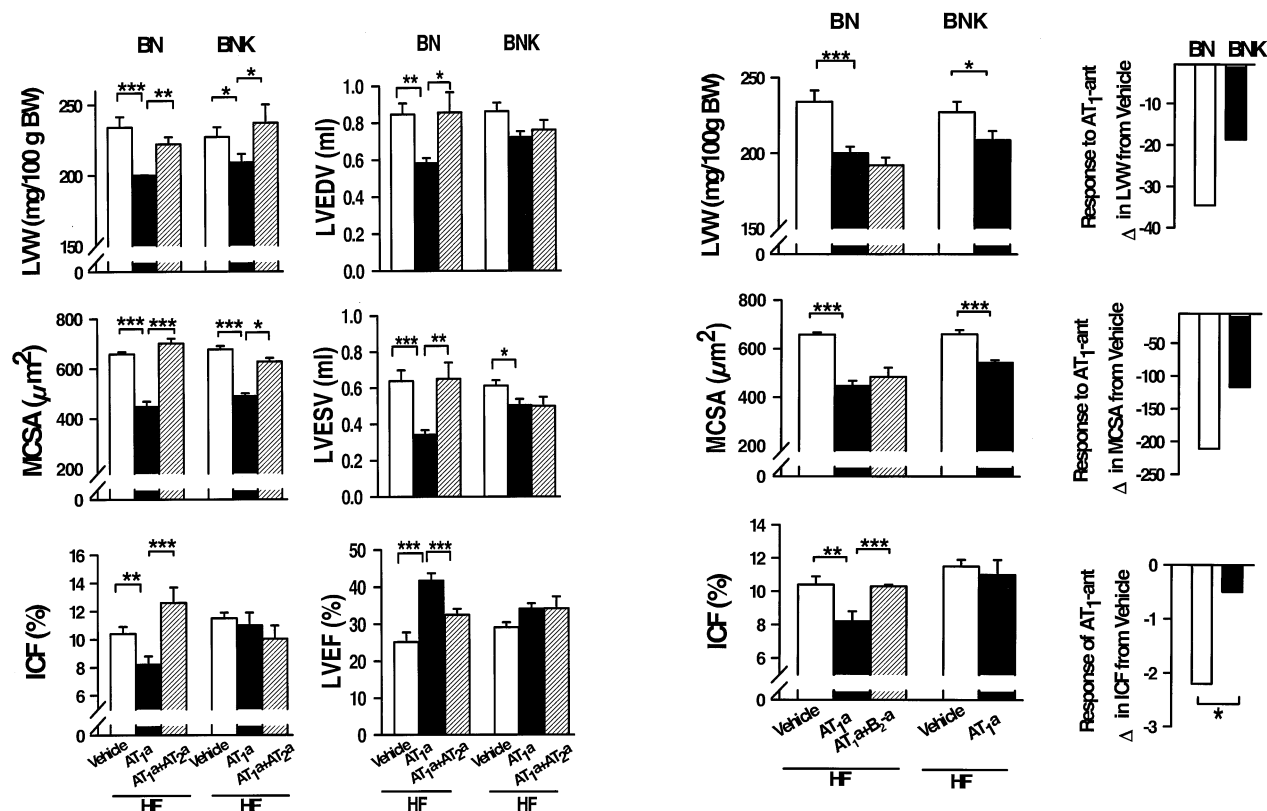


Figure 2. Effect of the AT₁-ant L158809 (AT₁a) alone and combined with the AT₂-ant PD123319 (AT₂a) on LVW, MCSA, ICF, LVEDV, LVESV, and LVEF in BN and BNK rats with HF after myocardial infarction. **p* < 0.05; ***p* < 0.01; ****p* < 0.001. Abbreviations as in Figure 1.

Figure 3. Effect of the AT₁-ant L158809 (AT₁a) alone and combined with the kinin B₂-ant icatibant (B₂) on LVW, MCSA, and ICF. **Right panels** show the difference in the response to AT₁-ant (Δ from HF vehicle) between BN and BNK rats with HF after myocardial infarction. **p* < 0.05; ***p* < 0.01; ****p* < 0.001. Abbreviations as in Figure 1.

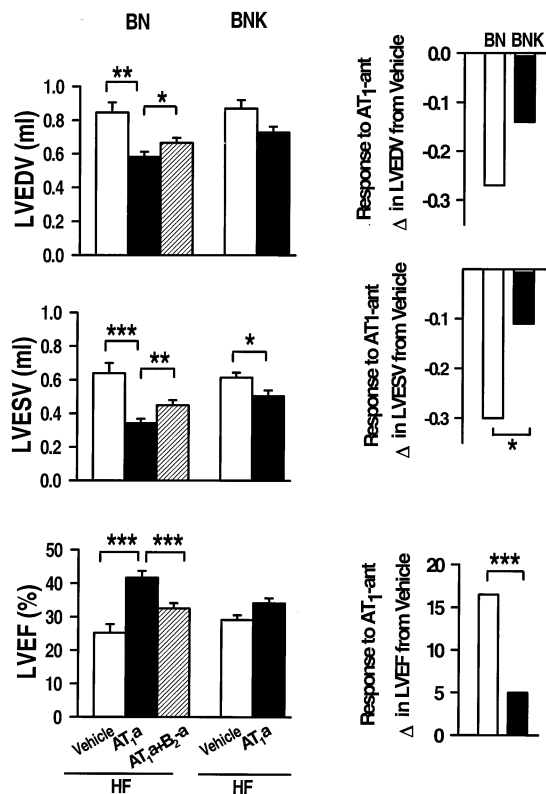


Figure 4. Effect of the AT₁a alone and combined with the kinin B₂ant on LVEDV, LVESV, and LVEF. **Right panels** show the difference in the response to AT₁-ant (Δ from HF vehicle) in BN and BNK rats with HF after myocardial infarction. *p < 0.05; **p < 0.01; ***p < 0.001. Abbreviations as in Figure 1.

onist blocked these beneficial effects but not the changes in MBP. In BNK rats, AT₁-ant decreased MBP as well but had no effect on function or cardiac remodeling. Thus, these data suggest that the cardioprotective effects of AT₁-ant in HF are independent of its effect on blood pressure reduction. Collectively, the present data support the hypothesis that when the AT₁ receptor is blocked, activation of the AT₂ receptor contributes to the cardiac therapeutic effects of AT₁-ant and, moreover, that the AT₂ receptor may act via two different mechanisms, one that is mediated by kinins and another that is not (Fig. 5).

There is now convincing evidence that AT₁-ant are effective in the treatment of HF (8,23). The mechanisms by which AT₁-ant exert their beneficial effects on HF in vivo are more complex than previously understood. Clearly, AT₁-ant prevent the effects of Ang II on the AT₁ receptor, and this antihypertensive effect may lead to cardioprotection (24); however, evidence is accumulating that the AT₂ receptor also plays an important role in the therapeutic effects of AT₁-ant. AT₂ receptors are expressed at low concentrations in normal cardiac myocytes but become upregulated in pathological states (3,5,25). It has been suggested that AT₂-ant may counterbalance the effects of AT₁-ant on cell growth and proliferation (26–28). Recently, Yang et al. (29) reported that overexpression of the cardiac AT₂ receptor improves baseline LV systolic function

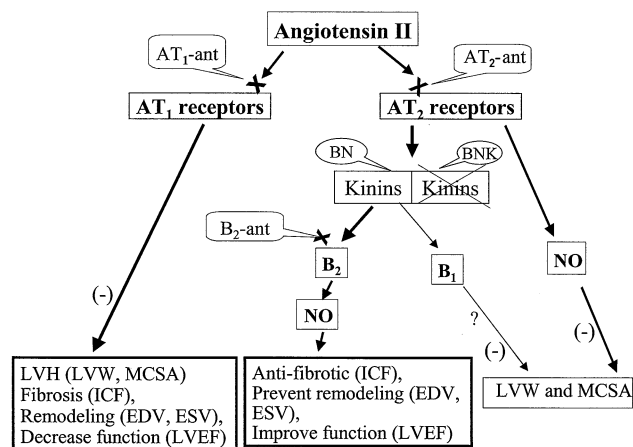


Figure 5. Hypothetical mechanism of action of AT₁-ant in heart failure: 1) AT₁-ant prevent the effect of angiotensin II on the AT₁ receptor, leading to cardioprotection (preventing cardiac hypertrophy, fibrosis, and remodeling and improving function); 2) angiotensin II activates the AT₂ receptors, which act via release of kinins, and B₂ and/or B₁ receptors, which act via release of nitric oxide, thereby leading to anticardiac fibrosis, preventing remodeling and improving function; a) in BN rats with HF, B₂-ant only blocks the B₂ receptor; the B₁ receptors may be activated and prevent cardiac hypertrophy; and b) in BNK rats neither B₁ nor B₂ receptors are activated; as circulating kinins are absent in this strain, and AT₂ receptors may act directly via nitric oxide to prevent cardiac hypertrophy. **Rectangular callouts** (AT₁-ant, AT₂-ant or B₂-ant) point to the receptor blockade site, and **oval callouts** point to two strains (BN and BNK). Abbreviations as in Figure 1.

and preserves function during remodeling after MI. We have recently shown that cardiac hemodynamic, functional, and histological phenotypes are similar between AT₂ knockouts and wild-type mice under basal conditions and during the development of HF; however, the benefits of AT₁-ant are attenuated in AT₂-KO mice, suggesting that AT₂ receptors are not essential for regulation of cardiac function and morphology, whereas they play a significant role in the therapeutic effect of AT₁-ant (30). Our data from BN rats in the present study show that AT₁-ant improved cardiac function and attenuated interstitial fibrosis, hypertrophy, and LV volume, while AT₂-ant blocked these protective effects, indicating that AT₂ receptors mediate the cardioprotective effect of AT₁-ant.

Brown Norway Katholiek rats are a substrain of BN rats with a spontaneous genetic mutation that impairs transportation of kininogen out of the cells where kinins are released from high- and low-molecular-weight kininogens by plasma and tissue kininogenases such as kallikreins (9,31). Evidence suggests that a local kallikrein–kinin system exists in the heart that enables it to synthesize and release kinins (10,11,32). Kinins act via two subtypes of G-protein-coupled receptors, B₁ and B₂ (33). B₂ receptors are constitutively expressed in most tissues and are responsible for most known effects of kinins (34); in addition, they are specifically inhibited by the B₂-ant icatibant. The B₁ receptor is only weakly expressed under physiological conditions but is strongly induced under pathological conditions, such as inflammation or tissue damage (35). Recent evidence suggests that the B₁ receptor may also play an important

role in pathological situations (12–14). In previous studies using a B₂-ant or B₂-KO mice, we speculated that in such cases kinins might act via the B₁ receptor. However, in BNK rats neither the B₁ nor B₂ receptor is activated because circulating kinins are absent in this strain.

Kinins appear to play an important role in some of the therapeutic effects of the AT₁-ant. The data in the present work with B₂-ant and in BNK demonstrate that the effects of AT₁-ant on fibrosis, LVEDV, LVESV, and LVEF were blunted by the B₂-ant and absent in BNK rats, suggesting that the benefits of AT₁-ant are partly mediated by the AT₂ receptor via kinins. In support of this hypothesis, Seyedi et al (36) showed in vitro that activation of AT₂ during blockade of AT₁ stimulated the release of autacoids such as prostaglandin E₂ and nitric oxide (NO) either directly and/or via stimulation of kinins. Tsutsumi et al. (37) reported that in aortas from mice with overexpression of the AT₂ receptor gene, Ang II caused a significant increase in kininogenase activity and cyclic guanidine monophosphate production, which was further enhanced by an AT₁-ant but blocked by an AT₂-ant, kinin antagonist, or NO synthase inhibitor. These findings suggest that AT₂ activation stimulates kinin release, which further promotes NO/cyclic guanidine monophosphate production in a paracrine manner and thus potentiates vasodilation and regional blood flow regulation. Furthermore, we recently demonstrated that the therapeutic effect of AT₁-ant on cardiac function and remodeling after MI was diminished in B₂ and endothelial nitric oxide synthase knockout mice, providing further in vivo evidence that kinins and endothelium-derived NO play an important role in the beneficial cardiac effect of AT₁-ant (17,38).

In summary, using BN and BNK rats with HF after MI, we have demonstrated that the cardioprotective effect of AT₁-ant is mediated by AT₂ receptors, which exert their actions (antifibrosis, function, and geometry) partially through kinins. Kinins play a lesser role in the antihypertrophic effect of AT₁-ant.

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